MEIOSIS IN POLLEN MOTHER CELLS OF STRAINS OF OENOTHERA PRATINCOLA BARTLETT

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

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MEIOSIS IN POLLEN MOTHER CELLS OF STRAINS OF OENOTHERA PRATINCOLA BARTLETT[1]

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(WITH PLATES VII–IX)

Introduction

Investigations of the nuclear behavior in the pollen development of *Oenothera lamarckiana* and certain of its derivatives, together with some of the other large-flowered, generally cross-pollinated species of *Oenothera*, have made clear some of the reasons for the peculiar genetical behavior that characterizes this genus, even though all the hypotheses proposed are in some respects at variance with one another. So far, few cytological investigations of the small-flowered, generally self-pollinated species have been made. There are papers by DAVIS (23), EMERSON (32), CLELAND (18, 19), and VALCANOVER (88); there are no cytological publications whatever on the types studied genetically by BARTLETT, FRIEDA COBB BLANCHARD, LARUE, KLAPHAAK, and MARAÑON at the University of Michigan. Of these, *O. pratincola* is the foremost in interest, due to the fact that it has been most intensively studied and has given rise to the most mutations. In addition to many genetical phenomena that are common with the large-flowered species, *O. pratincola* shows behavior peculiar to itself and to other small-flowered species (LARUE and BARTLETT 56, 57).

The strains of *O. pratincola* used in this research are those whose history has been published by BARTLETT (2, 3, 5) and COBB and BARTLETT (20). Although the eight strains of this species from Lexington, Kentucky are morphologically alike, the strain designated E has a genetical behavior differing from the other seven. This strain gave rise to variants in such large numbers that BARTLETT (4, 5) designated the phenomenon mass mutation. Some of the mutations produced by the other strains are also produced by strain E, but the latter is peculiar in throwing a series of forms with narrow,

[1] Papers from the Department of Botany of the University of Michigan, no. 298 Walker prize essay of the Boston Society of Natural History, 1928.

strongly revolute, thick-veined leaves, which do not occur in the other strains. Mut. *formosa*, an example in this series, has been completely described and illustrated (BARTLETT 5). Mut. *formosa* was chosen for this study because, being the most fertile of the revolute-leaved forms, it was most successfully used in crosses of genetical and cytological significance for the problems under discussion.

Oenothera pratincola strain C gives rise to mutations of several kinds in every generation (BARTLETT 3). This strain differs from strain E in that it has never shown mass mutation since coming under cultivation. Strain C is typical of the seven others, and was chosen for crossing with strain E and also with mut. *formosa*.

Strain M comes from a cross between mut. *formosa* and strain C, the former being the female parent. It was designated "M" because it shows Mendelian segregation. When mut. *formosa* is pollinated by strain C, the F_1 plants are all f. *typica*, that is, similar to *O. pratincola*. The F_2 generation splits into 3 f. *typica:* 1 mut. *formosa* (COBB 21). This strain becomes of great cytological and genetical interest because simple cases of Mendelian inheritance in *Oenothera* are uncommon. The first described case of Mendelian inheritance is afforded by *O. brevistylis*, which acts in crosses as a recessive with *O. lamarckiana* and even with unrelated species (DE VRIES 73, DAVIS 25, 26). The *brevistylis* complex, when introduced into the form *nanella-brevistylis*, is also recessive; when this plant is crossed to *nanella* and to *lamarckiana* it segregates in the F_2 in proportions close to the 1:3 Mendelian ratio (DAVIS 27). Another example showing Mendelian behavior is the dwarf mutation from *O. gigas* which also acts as a recessive in crosses with its parent (DE VRIES 92). It has been the contention of GATES (44, 45) that *O. rubricalyx* acts as a Mendelian dominant in crosses with *O. rubrinervis*, but SHULL (77) offers another interpretation. Another character, "old-gold," a flower color which arose as a gene mutation from *O. lamarckiana*, has recently been added by SHULL (80) to the list. In crosses with its parent *O. lamarckiana*, which has yellow flowers, this character acts as a Mendelian recessive.

Material and methods

The material on which this research is based was collected from pedigreed cultures in the Botanical Garden of the University of

Michigan, during the summers of 1925 and 1926. Various fixing fluids were used, of which only one (weak Flemming) failed entirely to give satisfactory results. One of Allen's modification of Bouin's solution (saturated aqueous solution of picric acid 75 cc., commercial formalin 25 cc., glacial acetic acid 5 cc., chromic acid 1 gm., and urea 2 gm.) gave excellent results. Allen's modification of Bouin's solution as used by CLELAND (16) was also very good. Strong Flemming with 1 per cent urea and 1 per cent maltose added, yielded very satisfactory fixations, as good as those of Allen's modification of Bouin's. Straight Bouin's solution also proved excellent. The fixations in chromo-acetic solution containing chromic acid 1 gm., glacial acetic acid 3 cc., and distilled water 200 cc. were generally good. When using strong Flemming it is necessary to wet and scrub the anthers quickly with a stiff hair brush, as suggested by DAVIS (23, 24), so as to loosen the waxy coat that envelops them, and thus facilitate quick penetration by the killing fluid. If this procedure is not followed with great patience and care, the fixations in strong Flemming are likely to be generally very poor.

Twelve fixations were made from four plants of *O. pratincola* strain E, six from three plants of *O. pratincola* mut. *formosa*, nine from three plants of *O. pratincola* strain C, and eight from four plants of *O. pratincola* strain M. Each fixation consisted of twenty-five buds.

Whenever a fixation was secured from a plant, pollen counts were made on four or five buds, picked at random, to determine the percentage of good pollen. The results of these tests are included in table I, and show for the four strains good pollen averaging about 86 per cent. Table I also gives the time at which fixations were made, the length of time the buds were left in the fixing fluid, the kind of fixing fluid used, and the results from various fixations.

When strong Flemming solution was used, the buds were left in the solution for two to three hours, and were then transferred for 24 hours to chromo-acetic solution, to avoid the excessive blackening of the material that ensues from the action of the osmic acid. In case of Bouin's solution and its modifications, three to six hours in the fluid gave excellent results.

All the stages of cell division were plentifully obtained in the

majority of fixations, made between 11:30 A.M. and 3:30 P.M. The material was dehydrated through grades of alcohol, cleared in xylol, and imbedded in paraffin. Longitudinal sections, varying from 6 to

TABLE I

QUALITY OF FIXATION AND TIME OF FIXING; POLLEN CONDITION OF PLANT

CULTURE	STRAIN	TIME OF DAY	HOURS IN FIXING FLUID	FIXING FLUID	QUALITY OF FIXATION	POLLEN GRAINS COUNTED	PERCENTAGE GOOD POLLEN*
68.1...	E	3:30 P.M.	3	Bouin modified	Excellent	350	90.3
68.9...	E	11:30 A.M.	4	" "	"	492	91.9
68.9...	E	12:30 A.M.	7	" "	"	543	85.2
68.1...	E	10:45 A.M.	6	" "	"	137	74.3
68.1...	E	1:45 P.M.	6	" "	"	260	85.0
68.9...	E	1:30 P.M.	5	Bouin	Good	129	84.9
68.4...	E	1:45 P.M.	28	Chromo-acetic	"	490	86.0
68.4...	E	12:5 P.M.	24	Strong Flemming	Excellent	114	82.5
68.1...	E	10:00 P.M.	24	" "	"	168	92.3
68.1...	E	2:00 A.M.	8	Bouin modified	Very good	182	81.9
68.1...	E	12:00 A.M.	7	" "	Excellent	351	92.4
68.5...	E	2:45 A.M.	26	Weak Flemming	Very good	108	86.4
63.6...	For-mosa	11:35 A.M.	5	Bouin modified	Excellent	220	89.5
63.8...	"	11:00 A.M.	7	" "	"	645	89.2
63.8...	"	4:15 P.M.	3	" "	"	320	87.4
63.6...	"	3:40 P.M.	4	" "	"	214	82.5
63.7...	"	1:45 P.M.	5	" "	"	113	83.5
63.7...	"	10:15 A.M.	7	" "	"	108	83.9
610.3...	C	12:20 A.M.	6	Bouin modified	"	64	93.4
610.3...	C	3:00 P.M.	24	Strong Flemming	"	78	76.2
610.3...	C	12:10 A.M.	6	Bouin modified	"	213	93.5
610.3...	C	11:45 A.M.	24	Strong Flemming	"	420	91.8
610.4...	C	4:20 P.M.	24	" "	"	205	82.9
610.3...	C	1:00 P.M.	6	Bouin modified	"	94	78.0
610.3...	C	1:35 P.M.	5	" "	"	150	84.7
610.1...	C	12:45 P.M.	24	Weak Flemming	Very poor	108	92.0
69.4...	M	11:35 A.M.	5	Bouin modified	Excellent	345	91.2
69.4...	M	4:30 P.M.	3	" "	"	219	90.5
69.4...	M	2:30 P.M.	5	" "	"	235	93.2
69.4...	M	12:55 P.M.	6	" "	"	400	90.1
69.2...	M	1:10 P.M.	7	" "	"	139	63.0
69.5...	M	1:20 P.M.	4	" "	"	321	87.8

* Average percentage of good pollen in strain E was 85.2; in mut. *formosa*, 86.00; in strain C, 86.5; and in strain M, 85.8.

11 μ, were cut and stained in iron-alum haematoxylin, which proved excellent for the sharp differentiation of the nuclear structures. All the important and critical stages in the development of the pollen were plentifully obtained from the various individuals from which the fixations were made.

MEIOSIS IN O. PRATINCOLA STRAIN E

MITOSES IN ARCHESPORIUM OF ANTHER.—The mitoses in the archesporium of the anthers of *Oenothera pratincola* strain E, conform generally to the account of *O. grandiflora* Solander given by DAVIS (22), and hence only the critical stages will be discussed.

Differentiation of the archesporium in the anthers of *O. pratincola* takes place at a very early period. Cells divide both lengthwise and crosswise during its development, and finally a single and sometimes a double row becomes transformed into pollen mother cells. Numerous nuclear divisions take place at the same time in various regions of the anther.

The sporophytic mitosis is best studied in the final mitoses of the archesporial cells, which generally convert the primary single row of cells into a double row that later becomes the pollen mother cells. The somewhat greater size of the nuclei, as compared with the nuclei of the other cells of the developing flower, facilitates accurate observation.

The diploid chromosome number in *O. pratincola* strain E and mut. *formosa* is fourteen, as usual for species of *Oenothera*. This can be seen clearly in the nucleus before the appearance of the spindle fibers prior to the metaphase of the last division in the archesporium. The chromosomes at this time appear as variously bent rods, as shown in the polar view of the equatorial plate presented in fig. 1. During the metaphase and the early anaphase the chromosomes assume the forms of thick U's and V's, due to condensation (fig. 2). In the late anaphase and with the approach of telophase, condensation proceeds still further. Because the chromosomes are so closely massed during the stages of late telophase, it was impossible to determine whether they undergo a definite lengthwise split into two parallel threads, as claimed by DIGBY (30) for *Osmunda* and FRASSER and SNELL (35) for *Vicia faba*, or whether they become irregularly alveolized and form together a continuous reticulum, as described by SHARP in *Vicia* (75) and *Tradescantia* (76). The nuclei then pass into the resting condition preliminary to meiosis, in which the outlines of individual chromosomes become entirely lost.

RESTING NUCLEUS BEFORE MEIOSIS.—The nuclei of the pollen mother cells pass through a relatively long period of rest before the

advent of the prophase of the heterotypic division. They measure 9–11 μ, and possess a prominent nuclear membrane. The most conspicuous structures in the nucleus at this time are the nucleoli, frequently three or four in number and sometimes as many as five and six. Only one of these bodies attains a large size (fig. 3), however, and sometimes a shining area resembling a vacuole may distinctly be observed within it. The large nucleolus is nearly spherical, and lies toward the center of the nucleus. The smaller nucleoli are scattered in the nucleus, and seem to have no definite positions. It is difficult to determine the exact number of nucleoli, since some of them are as small as the larger chromatin granules. Certain of the smaller nucleoli are frequently seen lying beside the largest, and these probably unite with the latter, as reported by GATES (43) in the case of *O. rubrinervis*.

During the resting stage the nucleus contains a chromatic reticulum of delicate single threads, evenly distributed through its interior (fig. 3). Very small chromatin granules may be seen irregularly scattered over these threads. At the juncture of two threads chromatin granules of varying shapes and sizes were distinctly observed. These fine threads run through peripheral regions of the nucleus and over the surface of the nucleolus, and are in direct contact with the chromatic material in the nucleolus (fig. 3). The threads are uniformly single and show no parallelism.

PRESYNIZESIS STAGES.—Shortly before the resting nuclei enter the prophase of the heterotypic mitosis, some parallelisms in the threads of the reticulum may be observed, but these are not numerous and seem not to be significant. It is quite natural that some parallelisms among the threads should exist, taking into consideration their number within so small a space. At the approach of the heterotypic prophase the threads of the reticulum begin to thicken and to contract gradually, and many of them unite with one another in an irregular manner. This process transforms the characteristic fine-meshed reticulum of the resting stage (fig. 3) into an open reticulum, the threads of which are coarser and rougher (figs. 4, 5). The union of threads with subsequent condensation which effects the transformation of a delicate reticulum into a system of coarse and rough threads, varying in diameter, is a gradual process (fig. 6).

While this is taking place, there may still be observed in the nucleus some delicate threads not yet thickened; and also some shriveled threads may be observed which appear to be in the process of delivering their chromatin content to the threads that are to survive. The chromatin accumulations that are scattered irregularly throughout the nucleus during the resting stage probably become amalgamated with the thickening threads, and even if they exist their presence is not recognized with certainty. The pull exerted on the peripheral threads, due to the contraction, shrinkage, and condensation of the internal threads, causes them to draw away from the nuclear membrane and they come to lie in a tangled mass at one side of the nucleolus, giving the characteristic stage of synizesis. Occasionally the threads very close to and connected with the nuclear membrane may still be seen in place, but they are not smooth and continuous (fig. 6). The nucleolus leaves its central position in the nucleus and moves to the periphery, where it generally lies flattened against the nuclear membrane, next to the contracted mass of threads (fig. 7).

Some parallelism among the threads may be observed at times during this process of condensation; but since the process is one of contraction, it is to be expected that threads will occasionally be drawn into side-by-side relations which are not to be regarded as significant.

The transformation of the fine-meshed reticulum, composed of single delicate threads with scattered chromatin granules of varying shapes and sizes, into a reticulum of rather large meshes, composed of threads of coarser and rougher nature, forming a spireme, has given rise to much discussion among prominent cytologists as to the method by which this transformation is brought about. Two interpretations have been offered for various material. One is supported by GRÉGOIRE (50, 51), ALLEN (1), ROSENBERG (69, 70, 71), STOMPS (85), and others and the other interpretation is held by FARMER and MOORE (33, 34), DIGBY (28, 29, 30), MOTTIER (65, 66, 67), FRASER and SNELL (35), FRASER (36), and their followers. Both schools agree that the most important event of this process in most material is a side-by-side association of threads during the earlier part of the prophase, but the two schools offer very different inter-

pretations of the history. The first interpretation, that of parasynapsis, holds that the two threads are homologous spiremes, one derived from the egg and the other from the sperm. These threads hold two sets of chromosomes, and their association gives rise to a bivalent spireme. The second interpretation, that of telosynapsis, maintains that the double thread when present is due to the lengthwise division of chromosomes during telophase, or even anaphase of the previous mitosis, and consequently does not represent an association of two spiremes of different descent. By the interpretation of telosynapsis, double threads, if found during the prophase, are merely associations of daughter spiremes.

The history of meiosis in *Oenothera pratincola* and all its strains favors the telosynaptic interpretation.

Synizesis

The thickening of the threads goes on for some time, due to contraction, and also because material is received from the chromatin granules and the other threads (fig. 6). Some of the threads become very thin, and certain of them become quite shriveled as their material presumably passes into the developing spireme. Certain parts of the threads near the nucleolus become gorged with material, appearing much swollen. Material from the nucleolus is apparently received by the nearest threads more rapidly than it is dispersed, and consequently parts of the threads become much thickened. The reticulum at this stage (fig. 6) shows a spireme composed of rather thick and uniform threads, some of which may be followed for a considerable distance in the tangled mass. The nucleolus loses its spherical shape and becomes somewhat flattened against the nuclear membrane. It is quite empty of chromatic material, as is seen by its reaction to the stain; but the endonucleolus is clearly seen as a very small round body taking the stain conspicuously (fig. 9). The spireme becomes more closely contracted, and finally has the appearance of a closely wound ball of threads, the synizetic knot (fig. 8). The knot remains in this much contracted condition of mid-synizesis for a long time, while the threads of which it is composed become more uniform in thickness and longer. The process of transformation from a reticulum of delicate threads into the spireme

that emerges at the end of synizesis takes place very slowly; in fact, synizesis is of longer duration than any other period in the development of the pollen. During certain stages the knot becomes so dense that it is impossible to determine with certainty what is happening within.

Open spireme

After some time the knot gradually begins to loosen, probably due to the lengthening of the threads. Many small loops of various sizes begin to extend from the periphery of the knot, while the greater portion of it is still so contracted that nothing of its structure can be distinguished with accuracy (fig. 9), although it is clear that it is composed of thicker threads. The loops on the periphery of the knot loosen while the central mass of threads is still drawn together in a very complicated manner (fig. 10). Sometimes during this stage the threads are so thick and full of chromatin that it is very hard to recognize them as threads. Finally they become uniform and those in the center loosen sufficiently to reveal their individuality. During no stage is the spireme entirely clear and free from chromatin accumulations, however, but differential staining shows it to be composed of distinct threads much swollen and gorged with material (fig. 11). During this emergence of the spireme from the contracted condition of mid-synizesis the threads become distributed uniformly throughout the nucleus. Some of them become very prominently beaded, the beads being placed in a single row, and these are probably the chromomeres of various authors (fig. 12). It is possible to trace a single thread for some distance as it winds about in the nucleus. No threads with free ends were found in this stage.

The nucleolus which lies flat against the nuclear membrane throughout synizesis and during the emergence of the open spireme later takes on a spherical form, and generally moves to the center of the nucleus. It is quite homogeneous and free from chromatic material, as indicated by its failure to stain; but the minute, deep-staining endonucleolus within may be distinctly observed (figs. 12, 13, 15).

The spireme has been carefully studied for evidence of double structure but none has been found; the threads are everywhere uniformly single.

During synizesis and the stages of the open spireme the pollen mother cells become round and separate from one another. In the stages prior to synizesis they are packed closely together and consequently are angular.

Second contraction

After remaining for some time in the open spireme stage, the nucleus enters the stage known as the second contraction. This stage is of relatively short duration. The threads that were rather widely and evenly distributed during the open spireme stage begin to contract, with accompanying condensation of chromatic material. The process of contraction and condensation takes place rapidly, and consequently the threads at the periphery come to form radiating loops from the central mass (fig. 13). The threads in the center become much swollen and form large irregular masses of chromatin (figs. 14, 15). The individual threads appear to be lost in the central mass, but differential staining shows this chromatic mass really organized, consisting of closely contracted and much thickened threads (fig. 13). It was not possible to determine whether these central threads unite with one another as they come to lie in a mass, but close observation indicates that they are distinct and separate, although very much swollen (figs. 14, 15).

As condensation proceeds, the material from the radiating loops is drawn to the center, and at the time of the greatest contraction the contents of the nucleus look like an irregular mass of chromatin with very slight traces of loops at the periphery. In no case, however, are these loops lost during any stage of second contraction. As the loops become shorter and shorter their two sides approach, until in some cases they actually come to lie against each other. Very soon there appear constrictions in the loops, which probably indicate segment of the spireme that are to become the chromosomes. Some loops are made up of only one chromosome (fig. 14), while others may have as many as two or three (fig. 15). After some time the outlines of the chromosomes become clear. While some of them may be distinguished clearly in one part of the contracted mass, it frequently happens that in other parts definite organization cannot be recognized (fig. 15). The formation of the chromosomes through segmentation of the spireme is easily demonstrated.

A most careful study failed to show any evidence of lengthwise fission among the loops in the spireme during the second contraction; the spireme was found to be univalent in nature, the threads being everywhere single. A split in the spireme would be expected if it were bivalent, since it is at this stage that the chromosomes prepare to separate from each other; therefore it is certain that each of these loops represents one or more chromosomes attached end to end.

The nucleolus, during the stage of second contraction, generally lies against the nuclear membrane, and is oval in form with little or no stainable material. In some nuclei it comes to the center. The endonucleolus may be observed at this stage as a black body inside the nucleolus (figs. 13, 15), which is not at all involved in the processes of second contraction.

Final prophase stages

Ordinarily in plants and animals the term diakinesis is used to denote the stage when the paired homologous chromosomes lie in the nucleus prior to the formation of the heterotypic spindle. It is doubtful whether this term should be used at all in the case of *Oenothera pratincola*, which shows no pairing but a closed chain of fourteen chromosomes attached end to end. During the prophase stages so far discussed there is little opportunity for studying the individual chromosomes, because of the complexity of the stages through which they pass, and also due to the fact that they are not in a compact form; but from now on stages will be dicussed in which individual chromosomes are clearly shown.

At the end of the second contraction there emerge gradually chromosomes, irregular in outline and connected end to end by delicate threads. They sometimes lie over one another in a confused mass, and frequently their arrangement cannot be determined with accuracy (fig. 16). Later they become more evident with the loosening of the contracted mass (fig. 17). Shortly after this stage the whole situation clears, and a very striking arrangement of the fourteen chromosomes attached by delicate threads end to end in a closed chain is readily observed (figs. 18–20). Sometimes an attachment breaks and instead of a closed chain of fourteen chromosomes an

open chain results (fig. 21). It is difficult, because of the length of the chain of chromosomes and because of its loops, to find sections in which all the members are present, all the attachments undisturbed and perfectly clear. I have examined nearly one thousand cells in this species and found no case of chromosomes in a paired side-by-side arrangement. The results of a critical examination of the nuclei at this stage are summarized in table II. Although many nuclei had been cut, so that certain chromosomes were missing,

TABLE II

ARRANGEMENT OF CHROMOSOMES DURING LATE PROPHASE IN OENOTHERA PRATINCOLA

ARRANGEMENT	NUMBER OF NUCLEI OBSERVED
Closed chain of fourteen chromosomes	37
Open chain of fourteen chromosomes (attachment broken at one point)	17
	Total 54
Thirteen chromosomes in chain, one missing	19
Twelve chromosomes in chain, two missing	31
Eleven chromosomes in chain, three missing	44
Ten chromosomes in chain, four missing	58
Nine chromosomes in chain, five missing	35
Eight chromosomes in chain, six missing	30
	Total 217
	Total 271

the observations uniformly showed that all the chromosomes present were in a continuous chain or ring.

This final stage of the heterotypic prophase lasts only for a short time. The nucleolus, pressed against the nuclear membrane, quite free of the chromatic material, disappears slowly. The deep-staining endonucleolus persists even after the disappearance of the nucleolus (fig. 23).

HETEROTYPIC METAPHASE

The nuclear membrane breaks down slowly. Its dissolution is first indicated by the surrounding cytoplasm becoming rather dense and by the development of delicate fibrils. The fibrils are at first scattered without order. When the nuclear membrane has complete-

ly dissolved they appear to penetrate the nuclear cavity, and very soon a multipolar spindle is formed with the fibers arranged in cones (fig. 23). The fibers enter the cluster of chromosomes in an irregular and complicated manner. The chromosomes, which have an irregular outline during the final stages of prophase, change in shape and size with condensation, becoming quite compact, when they look like thick V's and rods. The closed chain of fourteen chromosomes still persists (fig. 22), except when occasionally a delicate attachment breaks and an open chain results (fig. 23). The endonucleolus may be observed even at this stage, but with the approach of anaphase it disappears.

The multipolar spindle becomes bipolar, and the chromosomes, still in a closed chain, pass to the equatorial region of the spindle. Shortly after, the spindle fibers, which by this time are organized into clusters, attach themselves to the mid-region of the V-shaped chromosomes in such a way that the alternate chromosomes go to the same pole (fig. 24). Some of the chromosomes are rod-shaped, in which case the spindle fibers are attached to their ends. In view of the fact that all the fourteen chromosomes are attached to one another end to end in a chain, which is sometimes very much looped, it is difficult to obtain a clear view of all the chromosomes and their attachments. In spite of these difficulties, a thorough study was made of the best nuclei at this stage, so as to determine the exact nature of the attachments of the spindle fibers to the chromosomes. Only the nuclei presenting a clear view of all the fourteen chromosomes with their attachments have been included in this survey, a summary of which is given in table III. Hundreds of cells have been examined in which some of the chromosomes were missing but the attachments present were intact; these have not been included in table III.

The data presented in table III clearly show that the arrangement of the chromosomes in many nuclei during the heterotypic metaphase is zigzag. As a result of such an arrangement, alternate chromosomes go to the same pole in the species under discussion. Certain irregularities in the zigzag arrangement do occur, however, the percentage of which is greater in this strain than in *O. biennis* and "*O. muricata*," as reported by CLELAND (**18, 19**). It is 30.1 per cent for *O. pratincola* strain E. The irregularities may conveniently

be listed under the following headings: (1) Two chromosomes in different parts of the chain are suspended between the poles (fig. 25 *a*, *b*). If both of these go to the same pole, the distribution will be eight chromosomes to one nucleus and six to the other. In case they go to different poles the distribution will be seven chromosomes to each nucleus. (2) Two adjacent chromosomes in one part of the chain are attached to the spindle fibers leading them to the same pole; and in the other part of the chain a corresponding pair is similarly attached to fibers that lead them to the other pole (fig. 26 *a*, *b*). In

TABLE III

RESULTS OF SURVEY OF CELLS DURING HETEROTYPIC METAPHASE SHOWING CHROMOSOME ARRANGEMENT

CHROMOSOMES ALL DISTINCTLY VISIBLE AND END-TO-END ATTACHMENT CLEAR	NUMBER OF NUCLEI OBSERVED
Closed chain of fourteen chromosomes complete and regularly zigzag	135
Open chain of fourteen chromosomes complete and regularly zigzag	39
	Total 174
Closed chain of fourteen chromosomes complete and attachments irregular	43
Open chain of fourteen chromosomes complete and attachments irregular	32
	Total 75
	Total 249

this type of irregularity, however, the number of chromosomes going to the two poles will be equal, seven to each pole. (3) A pair of adjacent chromosomes is drawn to the same pole, while a chromosome is uncertainly suspended in the equatorial region (fig. 27 *a*, *b*). This chromosome might go to either pole. If it is drawn to the pole with the abnormally placed pair, the number of chromosomes passing to that pole will be eight and the other pole will then have only six chromosomes. All these irregularities have been noted by CLELAND (**18, 19**) for *O. biennis* and "*O. muricata*." (4) Three or sometimes four chromosomes in a group have fiber attachments of such a nature that it is impossible to predict the number that might go to the two poles (figs. 28–30).

Careful observation during mid-interkinesis following the hetero-

typic mitosis also gives some idea of the irregularity in the chromosome distribution. Nine hundred nuclei in this stage were examined, of which ninety-four were found to have eight or six chromosomes, which is an irregularity of 9.4 per cent. Since irregularity in the zigzag arrangement during metaphase is not necessarily the only cause of abnormal distribution (six and eight) of the chromosomes, the total percentage of irregularities is higher, and was actually found to be about 30.1 per cent.

Heterotypic anaphase and telophase

In early anaphase the chromosomes present the zigzag arrangement (fig. 24) which is so clearly described and illustrated by Cleland in all his papers on the cytology of *Oenothera*. The chromosomes separate during anaphase into two sets, breaking their connection with each other. Presently they reach the two poles of the spindle, seven passing to each pole when the distribution is regular (fig. 31), and eight to one pole and six to the other if the distribution is irregular (fig. 32). Soon after the chromosomes reach the pole they generally form a small star-shaped cluster, with one chromosome in the center and six at the periphery, an arrangement typical of species of *Oenothera* at this stage (fig. 33). After some time the chromosomes split lengthwise (fig. 34), the split at first not being conspicuous. The chromosomes spin out delicate threads which generally connect them with one another. A vacuole-like space appears around the group of chromosomes, and the outer boundary of this area marks the nuclear membrane.

Interkinesis

The slender threads connecting the chromosomes become hazy during the interkinesis and the nuclei enlarge rapidly. The chromosomes also enlarge and become irregular in form. Shortly after this the split in the chromosomes becomes conspicuous, and the halves frequently separate to give an irregular X (fig. 35). These halves or daughter chromosomes are loosely connected in their middle region. In certain cases the chromosomes come to show denser regions within, which stain more deeply than the main body of the chromosomes (fig. 35).

During this stage one or more nucleoli appear at first as minute globules, in connection with chromosomes. Sometimes they are associated with the chromatin threads that pass between the chromosomes (fig. 34). It appears that they arise *de novo*, as there is nothing to indicate their survival from previous stages.

Homeotypic division

The daughter chromosomes which arise from the lengthwise division of the heterotypic chromosomes condense during the prophases and finally appear as short rods (fig. 36). As soon as the nuclear membrane breaks down, the spindle fibers become conspicuous and enter the nuclear cavity. Very soon a multipolar spindle is formed, which later becomes bipolar, and the chromosomes pass to the equatorial region of the spindle (fig. 37). The fibers, which are organized by this time into thick clusters, attach themselves to the perfectly paired daughter chromosomes. Shortly after this the daughter chromosomes separate and pass to the poles, seven chromosomes in each set. The homeotypic division is normal except where there has been an irregularity in the preceding heterotypic mitosis, in which case eight and six chromosomes respectively may be distributed instead of the normal seven (fig. 36).

The four groups of chromosomes resulting from the homeotypic division lie rather close together in the pollen mother cell. Shortly, a clear hyaline space resembling a vacuole arises around each group. The outer boundary of this vacuole ultimately becomes the nuclear membrane. The nuclei then enlarge rapidly, the chromosomes gradually becoming long and irregular in form, and they spin out delicate threads which later become irregularly thickened. Gradually the chromatin material from the chromosomes becomes located in these threads. The outline of the chromosomes may still be recognized at this stage, but as the dispersion of the chromatin proceeds the boundaries of chromosomes can no longer be recognized. Secondary threads arise from the primary threads, and the chromatic material passes rapidly into them. Finally the nucleus becomes filled with a network of irregularly thickened threads, as shown in fig. 38. Later, one or more nucleoli appear together with chromatin granules of varying shapes and sizes.

Meiosis in O. pratincola mut. formosa

The nuclei of the pollen mother cells in mut. *formosa* during all the stages of meiosis and the following division resemble those in *O. pratincola* strain E.

Meiosis in O. pratincola strain C

The history of meiosis in strain C resembles that of strain E and mut. *formosa* in all respects. Careful observations were made on hundreds of nuclei of the pollen mother cells in this strain, in the hope of discovering some peculiarities distinguishing it from strain E and from mut. *formosa*, but nothing different was found. It may be stated confidently that if the slides of strain E, strain C, and mut. *formosa* were mixed it would be impossible to distinguish them.

Meiosis in O. pratincola strain M

It will be remembered that strain M is the cross mut. *formosa* pollinated by f. *typica* of strain C. It has the appearance of *O. pratincola* f. *typica*, the flat-leaved character of which dominates the revolute-leaved character of mut. *formosa*. The F_1 plants cannot be distinguished from strains E and C of *pratincola*, therefore, although they carry the revolute-leaved character of mut. *formosa* as recessive.

When self-pollinated, strain M gives an F_2 progeny showing simple monohybrid segregation of 3 typical *pratincola:* 1 mut. *formosa*. The strain was designated M by BLANCHARD (COBB **21**) because of the Mendelian segregation.

All the stages in the course of meiosis in strain M show clearly that there are no fundamental differences between this form and the other strains discussed up to and including the second contraction. With the unfolding of the irregular threads of the second contraction, however, an entirely new situation is presented. All the strains discussed before show chains of fourteen chromosomes attached end to end in the later stages of prophase. Strain M has a closed chain of twelve chromosomes attached end to end, to which is linked a pair of chromosomes in the form of a ring (fig. 41 *a*). In the earlier periods of metaphase the ring of two chromosomes remains attached to the circle of twelve (figs. 42 *a*, 43 *a*), but during the late metaphase it breaks away and takes a position at one side of the spindle

(fig. 44 *a*). The chromosomes assume the form of thick V's and rods, due to condensation. The characteristic zigzag arrangement is clearly shown in the closed chain of twelve chromosomes, alternate chromosomes passing to the poles of the spindle. The chromosomes of the detached pair separate and accompany the two groups of six chromosomes as they pass to the poles. The chromosomes from the pair may be followed into late anaphase, but with the approach of the telophase each becomes associated with the other six chromosomes, and it is impossible to distinguish them. The pair of chromosomes is significant of the genetic behavior of strain M. These homologous chromosomes probably carry respectively the genes for flat leaf (*pratincola*) and revolute leaf (*formosa*), and their segregation and recombination in the breeding of strain M accounts for the simple Mendelian ratio peculiar to this monohybrid. The remaining stages of pollen formation in this plant are the same as those described in *Oenothera pratincola* strain E.

Formation of pollen grains

The four microspore nuclei grow rapidly and delicate fibers appear between them. These sometimes become prominent (fig. 45), resembling spindle fibers, but they soon disappear. Shortly small refractive vacuoles begin to develop in the cytoplasm (fig. 46). These are numerous at first, and distributed rather uniformly through the cytoplasm of the pollen mother cell. The regions of the mother cell between the nuclei infold at the periphery (fig. 46), nearly equidistant from the nuclei and at right angles to the former spindles; so that when the new walls are developed the resulting spores are tetrahedral in form. Later, vacuoles become arranged in the cytoplasm between the nuclei and enlarge, some of them fusing to form large flattened vacuoles (fig. 47). They finally become plates which extend from the center of the cell between the nuclei to the infolded regions at the periphery. The complete cleavage of the protoplasmic material appears to result from the furrows originating at the surface (fig. 48), which grow inward very rapidly, breaking through the large vacuoles. The furrows are thin at first but later become wider and conspicuous.

C. H. Farr (37, 38, 39, 40) has shown that the simultaneous divi-

sion of the pollen mother cells in *Nicotiana, Magnolia, Sisyrinchium*, and *Nelumbo* occurs by means of furrows. Before this time it was thought that the quadripartition was brought about in conjunction with cell plates formed between spindles. W. K. FARR (41) also reports a similar process in *Coboea*, and CASTETTER (12, 13) for *Melilotus* and *Cucurbita*. CLELAND (15, 16, 18, 19) has noted an invagination process, which he does not fully describe, in *Oenothera franciscana, O. biennis, O. biennis sulfurea*, and *"O. muricata."* The present studies on *Oenothera pratincola* agree with his observations so far as he records them. He does not refer to the vacuolation which precedes the final wall formation.

A membrane appears very soon around each young microspore, first recognized as a delicate film lining each cell cavity of the tetrad. It is distinct from the wall of the pollen mother cell although in most intimate contact with it; this membrane presently thickens. The young pollen grain is bluntly tetrahedral, with the peripheral side infolded to form a basin. The cell cavity is densely filled with protoplasm and there are no large vacuoles. The concavity of the basin is directed toward the apex of the tetrahedron. The wall is extremely thin at the three peripheral angles of the pollen grains. Shortly it thickens considerably and a mucilaginous material develops at these three angles. The little disks of mucilage enlarge and extend somewhat laterally. FRITSCHE introduced the name "Zwischenkörper" for these mucilaginous disks, and the same name has been used by NÄGELI and STRASBURGER. BEER (7) calls them interstitial bodies. The normal pollen grains of *O. pratincola* have three of these interstitial bodies. Some have only two and still others have only one. These cases are probably abnormal. The pollen grains of *gigas* forms of *Oenothera* have four or more of these disks. Very soon the wall of the pollen mother cell breaks down and the pollen grains are set free. As the pollen grain enlarges, the three interstitial bodies become more and more prominent, finally becoming three lobes. This gives the peripheral face of the pollen grain a more pronounced triangular appearance. Shortly a secondary thickening develops within the first pollen wall, extending over the whole of the inner face of the pollen membrane but very thin at the three lobes. A disk-like partition is formed across the base of each lobe. This disk con-

sists of two parts, the outer part a dense homogeneous layer and the inner part a less dense stratified lamella which is cap-shaped. The cavities of the three lobes at this stage no longer contain the mucilaginous substance which earlier takes a brilliant stain with haematoxylin. BEER (7) suggests that this mucilaginous substance is used in the formation of the closing disks. He thinks that the closing disk is later eaten away by a solvent, probably an enzyme secreted by the protoplast. The thickening of the pollen wall continues in the regions between the lobes. My observations on the development of pollen in *O. pratincola* are in accord with those of BEER for *O. biennis* and *O. longiflora*.

Cytological discussion

The conclusions of GATES (42, 43, 46), DAVIS (22, 23, 24), GEERTS (48), CLELAND (15, 16, 18, 19), VALCANOVER (88), and HÅKANSSON (53) are all in substantial agreement that the arrangement of the chromosomes in *Oenothera* is telosynaptic. Only one investigator, BOEDIJN (9, 10), maintains it to be parasynaptic, and his observations are at variance with those of the investigators just cited. BOEDIJN reports for *Oenothera lamarckiana*, during the early part of the heterotypic prophase, the side-by-side conjugation of the thick threads. From this stage he passes to the stage known as the second contraction, and figures pairs of long slender threads (two chromosomes) twisted in such a manner as to give the appearance of normal strepsinema stage. In early diakinesis stages he observed seven pairs of chromosomes, often entirely separate from one another and sometimes forming rings, and states that the single chromosomes comprising these pairs may split partially during this stage. SINOTÔ (81) reports the pairing of homologous chromosomes in *O. lamarckiana*. He does not think that they are parasynaptically arranged (82) after the beginning of the prophase in *O. sinuata* L. CLELAND (17) finds in *O. lamarckiana* at diakinesis a circle of twelve chromosomes and a ring of two, in contrast to the seven pairs described by BOEDIJN at this stage. GATES (42), GEERTS (48), DAVIS (24), and HÅKANSSON (53), who have also worked on the cytology of *O. lamarckiana*, do not report any evidence that would lead one to believe in the parasynaptic interpretation proposed by BOEDIJN.

It is worth noting here that some genera related to *Oenothera* have been stated to show parasynaptic arrangement of the chromosomes. TÄCKHOLM (86) for *Lopezia*, MICHAELIS (64) and SCHWEMMLE (72) for *Epilobium*, HÅKANSSON (52) for *Godetia*, and SCHWEMMLE (73) for *Eucharidium* report parasynapsis. SCHWEMMLE (72) reported telosynapsis in *Oenothera*, but changed his interpretation through conclusions reached in a cytological study of *Eucharidium concinnum* (73). He describes for *Eucharidium* three methods of gemini formation, one of which (Reihe C) resembles that of *Oenothera* in some stages. His figures, apparently of second contraction (figs. 27–29, Taf. V), resemble those of CLELAND for this stage (15 figs. 15, 21; 16 fig. 5). The interpretations put on apparently similar figures by the two investigators, however, are different. CLELAND and others believe that the chromosomes that emerge from second contraction result from the segmentation of a univalent spireme. SCHWEMMLE, on the contrary, maintains that during the second contraction the chromosomes are already in pairs. He gives some figures (figs. 2–5, 12–14, 20–22) of the stages prior to second contraction. These stages are critical for the determination of telosynapsis or parasynapsis in any species. None of these figures resembles the stages that I have observed in *Oenothera pratincola*, nor do they resemble the figures of other investigators of *Oenothera* cytology, with the possible exception of certain of BOEDIJN'S (10) figures of the early prophase. The reported absence of these stages in *Oenothera*, which according to SCHWEMMLE determine telosynapsis or parasynapsis, is believed by him to be due to environmental conditions, such as temperature and other undecided factors, which suppress their appearance. He suggests that as a result of these conditions the normal development of the anther and the pollen mother cell is in some cases retarded and in other cases prolonged. He predicts that it may become possible to demonstrate parasynapsis in *Oenothera*. It must be remembered that *Eucharidium* and *Oenothera* are distinct genera, and that conclusions for *Oenothera* through a study of *Eucharidium* may not hold good for the former.

In a recent paper, SCHWEMMLE (74) reports on the cytology of the cross *Oenothera berteriana*×*Oenothera* (*Onagra*) "*muricata*." Both parents have fourteen chromosomes, but those of the latter are twice

as large as those of *berteriana* (as observed during the early anaphase). All the stages of meiosis in this hybrid, from the heterotypic prophase to the untangling of the second contraction, are similar to the stages usually met in the meiosis of species of *Oenothera*. At diakinesis the chromosomes of "*O. muricata*" form a closed chain, as shown by CLELAND (19); the arrangement of the chromosomes in *O. berteriana* at this stage is not known. SCHWEMMLE states that the hybrid shows great variation in the arrangement of the chromosomes at diakinesis. He reports in some nuclei two small chains of three chromosomes arranged end to end, a pair of chromosomes, and six single chromosomes; in other nuclei many pairs; and in still others chains of chromosomes of large number accompanied by fewer univalent chromosomes. Cases of small chromosomes pairing with large ones are also reported by SCHWEMMLE. When the nuclear membrane dissolves, prior to metaphase of the heterotypic mitosis, six of the larger chromosomes of "*O. muricata*" may be recognized; but the seventh is not to be clearly seen (74). The small chromosomes of *O. berteriana* are likewise seen at this stage. The arrangement of the paternal and maternal chromosomes on the spindle at the heterotypic metaphase is variable. During the late metaphase, when some of the chromosomes have nearly reached the pole, others are seen lagging in the mid-region of the spindle. The distribution of the chromosomes to the two poles during anaphase is also variable. SCHWEMMLE observes the following cases: (1) four large three small to one pole and three large four small to the other; (2) five large two small to one pole and two large five small to the other; (3) six large one small to one pole and one large six small to the other; (4) seven large to one pole and seven small to the other. He finds abundant spindles with unequal chromosome numbers at anaphase, resulting in irregular distribution of the chromosomes to the two poles. He suggests that this irregularity is due to the fluctuations of the autumnal temperature when the material was fixed. He concludes that in a cross between two species of *Oenothera* presenting size differences in the chromosomes, the paternal and the maternal can be recognized and their distribution during meiosis observed.

The four strains under discussion, *O. pratincola* f. *typica* strain E, mut. *formosa*, f. *typica* strain C, and strain M, have been care-

fully examined and the arrangement of the chromosomes in all of them was found to be telosynaptic. All these strains will be treated together in this discussion because they show the same characteristic behavior. During the stages of the early heterotypic prophase there is hardly any evidence suggestive of parasynapsis. When the threads thicken and contract some of them may be found side by side, but such chance parallelism is to be expected. By careful and detailed observations it becomes evident that the formation of the spireme in the heterotypic prophase is effected by an irregular process of condensation, rather than by a side-by-side approximation of two distinct thread systems. During the open spireme stage following synizesis the threads are uniformly single, and there is no evidence of a bivalent spireme. During the second contraction following the open spireme stage, when the chromatin threads are thrown into radiating loops, these loops are also univalent. The most significant evidence that the chromosome arrangement in these strains is telosynaptic is presented with the unfolding of the chromatin threads at the end of the second contraction, from which emerges a closed chain of chromosomes attached end to end. There is no doubt that this chain is the outcome of the segmentation of the spireme at the termination of the second contraction stage.

The arrangement of the chromosomes in closed chains at the end of the second contraction stage, together with the extreme regularity with which all the stages are executed, leads CLELAND to conclude that the delicate threads of the resting nucleus in the species of *Oenothera* which he has investigated give rise to chromosomes definitely placed end to end and not to chance arrangements.

In most plants and animals the homologous chromosomes at the stage termed diakinesis are distributed in pairs throughout the nucleus. In *Oenothera* the situation at this stage, with the exception of a few species, is different. In most of the species of *Oenothera* which have been cytologically studied, a closed chain of some or of all of the chromosomes is found. Various types of chain formation have been described. In *O. franciscana* CLELAND (15) reports a closed chain of four chromosomes and five pairs; the chain of four, however, breaks into two pairs of chromosomes at the metaphase; in *O. biennis* and *O. biennis sulfurea* CLELAND (18) and EMERSON (32) ob-

served no pairing at all but two chains, one of eight chromosomes and the other of six, an arrangement which VALCANOVER (88) and KIHARA (54) confirm for *O. biennis*. In both *O. franciscana sulfurea* and *O. lamarckiana* CLELAND (16, 17) found a chain of twelve chromosomes to which is attached a ring of two chromosomes. His finding is confirmed by HÅKANSSON (53) for *O. lamarckiana*. Finally, SCHWEMMLE (72) in *O. rosea* and CLELAND (19) in "*O. muricata*" discovered a closed chain of all the fourteen chromosomes. Only in *O. blandina* and *O. deserens* has CLELAND (17) found seven independent pairs of chromosomes. SCHWEMMLE (72) also found seven independent pairs of chromosomes in *O. hookeri*.

When chains of chromosomes are present it becomes a matter of great interest to determine how the distribution of their chromosomes to the two poles takes place. CLELAND holds that the zigzag arrangement of the chromosomes at the heterotypic metaphase explains the situation. He states that in all the species of *Oenothera* he has investigated, where there are chains, the alternate chromosomes of the chains, because of their fiber attachments, generally pass to the same pole during the heterotypic metaphase, thus effecting the segregation of the homologous chromosomes. VALCANOVER (88), HÅKANSSON (53), and KIHARA (54) observed the characteristic zigzag arrangement described by CLELAND at this stage. EMERSON (32) did not see the zigzag arrangement in *biennis* as described by CLELAND, and consequently did not believe that the alternate chromosomes go to the same pole. He reported that the separation of the chromosomes is irregular. He suggested that *O. biennis* has fourteen different chromosomes instead of seven pairs of homologous chromosomes, and the chromosomes of this species are not homologous at all because they do not pair.

In all the strains of *O. pratincola* and mut. *formosa* the chromosomes are distributed to the opposite poles in the characteristic zigzag manner described by CLELAND, with occasional irregularity however. Whether such a zigzag arrangement leads to the separation of the homologous chromosomes, as assumed by CLELAND, and whether the chromosomes in *Oenothera pratincola* are homologous cannot definitely be stated for the present. In light of the genetical behavior of these strains, the maternal and paternal sets of chromo-

somes come out of meiosis as they entered the zygote. The only exceptions are due to irregularities in the zigzag arrangement. It appears from the genetic behavior, to be discussed in the following section, that all the chromosomes in *O. pratincola* are not homologous.

As discussed, a closed chain of fourteen chromosomes is formed in the final prophase stages, both in f. *typica*, strain C, and mut. *formosa*. Following a cross between mut. *formosa* (revolute leaves) and strain C (flat leaves), the F_2 homozygous dominant (flat-leaved) shows at this stage not a closed chain of fourteen chromosomes, but a circle of twelve chromosomes and a ring of two chromosomes attached to it. The question that presents itself is why and how the closed chain of fourteen chromosomes breaks into a closed chain of twelve chromosomes and a ring of two. An interpretation offered by Dr. F. Cobb Blanchard is given in the genetical discussion to follow.

The nature and function of the nucleolus have been the subject of much discussion in older as well as in more recent cytological literature. Some investigators believe that the nucleolus is merely a secretion product of the nucleus. A great number of the older writers (for literature see Wager 93), as well as modern cytologists, such as Lenoir (58, 59), Litardière (60), Tamura (87), Van Camp (89), and many others believe, however, in the transportation theory. In *Oenothera*, Cleland (15, 16, 18, 19) and Boedijn (9) support the transportation theory. Sinotô (82), working with *O. sinuata* L., finds no evidence for the transportation theory; that is, finds no evidence that the nucleolus acts as a storage structure.

The nucleus of pollen mother cells of *O. pratincola* contains at least one large spherical nucleolus. Sometimes more than one may be present. During the late synizesis some of the threads connected with the nucleolus become greatly enlarged, as though material had suddenly been transferred to them. This suggests that the nucleolus acts as a reservoir, from which chromatic substance is drawn to build up the spireme in the final stages of the heterotypic prophase. The nucleolus in the resting nucleus stains very deeply with haematoxylin, but very little of the stain is held in later stages of prophase, probably because the stainable material is carried away to build up

other structures. When the nucleolus is divested of all of its coloring matter, it shows in its interior a very small spherical body, the endonucleolus. This minute structure is sometimes connected with the spireme, but in most cases lies freely in the interior of the nucleolus. It disappears during the late metaphase. During interkinesis many nucleoli develop in contact with the chromosomes, arising always *de novo;* hence it appears highly probable that the nucleolus is a storage organ for material, akin to chromatin, which is formed anew in each nucleus.

The segmentation of protoplasm by cleavage furrows was observed by Bütschli (11). He interpreted the process as the result of the higher surface tension at the equator of the cell caused by the flow of the protoplasmic currents toward the centrosome. McClendon (61, 62, 63), Speck (83, 84), and Chambers (14) corroborate Bütschli's theory by their own observations, and present strong evidence in support of it. Kite (55), however, maintains that cleavage is due to "concomitant shrinkage and swelling or change in water-holding power of the different portions of the cytoplasm." Gray (49) thinks that the cleavage furrow is due to an equilibrium established between the effect of the movement in the protoplasm and the surface tension on the surface of the cell. Castetter (12) agrees with Bütschli's theory and its supporters.

The division of the protoplasm to form the pollen grains in *O. pratincola* is a process of segmentation through cleavage furrows. Careful observation shows that the granular cytoplasm moves toward the nuclei from the regions of the protoplasm equidistant from the nuclei. This results in vacuolate hyaline regions between the nuclei. The smaller vacuoles fuse to form larger ones arranged between the nuclei. The cleavage furrows progress from the periphery very rapidly, meeting the vacuoles with which they fuse. The process of cleavage is greatly assisted by the presence of the vacuoles with which the furrows fuse as they progress inward.

Genetical discussion

In many species of *Oenothera* chromosomes do not pair at diakinesis; such is the case in *Oenothera pratincola.* The failure of chromosomes to pair in this genus is presumably due to lack of the

usual affinity resulting from differences in their genetic constitutions. Many explanations have been offered to account for this unusual phenomenon. According to CLELAND (18), chromosomes which pair in diakinesis are relatively homozygous and those which do not are relatively heterozygous. CLELAND (15, 16, 18) suggests that the chain formation is probably due to hybridity. HÅKANSSON (52), in his paper on *Godetia*, states that hybrids present at diakinesis a strong tendency to form circles. On the other hand, GATES (47) finds a regular pairing of the chromosomes in a hybrid of *Oenothera*. OEHLKERS (68) finds in *Oenothera* that there are hybrids with regular pairings as well as those with chains; hence the hypothesis that the formation of rings in certain species of *Oenothera* is an indication of their hybridity, seems untenable. CLELAND (18) also suggests two other ways in which the incompatibility of the chromosomes might have arisen. The first is that it may be due to gradual accumulation of gene mutations within a chromosome. This process, he thinks, is probably aided by the appearance of balanced lethal factors. The second is that, when the chromosomes are arranged end to end, it may be more difficult for them to pair than it is when the homologous chromosomes are placed side by side.

In explanation of the genetical phenomena which *Oenothera pratincola* exhibits, a hypothesis was formulated by BARTLETT (6) which assumes that two unlike types of gametes are produced, which he calls α and β. Generally the functional egg is an α gamete, and the functional sperm a β gamete, the β eggs and α sperms usually failing to function. When a zygote is formed in *O. pratincola*, therefore, it generally has the constitution $\alpha\beta$. It receives the α determiners of the female parent and the β determiners of the male parent. The results of intercrosses between the entire group of forms indicate that the differences in the characteristics of the several types are in general determined by the α gamete, and thus the modifications of the α gamete are responsible for the greater number of mutations in *Oenothera pratincola*. This is shown by the matroclinic inheritance which the mutations under discussion display in crosses with their parent form. In general, pollination by the β gamete of a mutation has the same effect as by a β gamete of f. *typica* from which the mutation arose. There are very few characters

that are affected by different sources of the β gamete. Among the mutations that have been genetically studied, probably there is no mutation of $\alpha\beta$ constitution effected by a change in the β gamete alone except the one reported by DE VRIES (91) in the case of *O. biennis sulfurea*. This mutation shows patroclinic inheritance in crosses with its parent.

According to BARTLETT'S hypothesis, the characteristic portion of the α and the β gametes may consist of a single chromosome or a group of chromosomes. The chromosomes of the α and the β groups do not segregate according to the law of chance in reduction division; instead they pass together as groups to the opposite poles. They are passed to the daughter nuclei as distinct sets, one from the maternal and the other from the paternal parent. In addition to the characteristic chromosome or chromosomes, there may be in some forms chromosomes which are freely segregating. These carry factors for characteristics which show Mendelian inheritance.

For the better understanding of the discussion that is to follow, it is rather important to understand thoroughly what is meant by homologous chromosomes. In most plants and animals, and some of the species of *Oenothera*, when the chromosomes pair during diakinesis, and probably carry allelomorphic factors, such chromosomes are referred to as homologous. In cases when the chromosomes do not pair, and are not known to carry allelomorphic factors, the use of the term homologous chromosomes is rather confusing, because of our present day conception of the term in connection with Mendelism. Some of the chromosomes of *O. pratincola* are not homologous.

When different strains of *Oenothera pratincola* are intercrossed they show a behavior which is very significant genetically. Mut. *formosa* (revolute leaves) is given by strain E (flat leaves). In crosses between the two forms the inheritance is matroclinic, as shown by BARTLETT (6) and COBB and BARTLETT (20). This behavior of mut. *formosa* shows that it arose from strain E through a change in the α gamete of E. When f. *typica* of strain C is pollinated by mut. *formosa*, the F_1 progeny consists of plants in appearance exactly like f. *typica*. All the F_2 plants resemble f. *typica*. If mut. *formosa* is used as the pistillate parent and f. *typica* of strain C as the staminate

parent, all the F_1 individuals are flat-leaved, resembling strain C. When these F_1 individuals are self-pollinated, they give in the F_2 generation a ratio of three flat-leaved plants to one having revolute leaves. This simple Mendelian segregation continues in the following generations, as shown by COBB (21). The flat-leaved hybrid strains from this cross (mut. *formosa* × f. *typica* strain C) have been designated as *O. pratincola* strain M by BLANCHARD (COBB 21), and one homozygous flat-leaved strain has been carried under that name for the last seven years. A closed chain of fourteen chromosomes is formed in the final prophase stages (diakinesis), both in mut. *formosa* and in strain C. The homozygous dominant strain M, however, shows at this stage a closed chain of twelve chromosomes to which is attached a pair of chromosomes. These two chromosomes are homologous and contain the factors for flat and for revolute leaves. Such a pair of chromosomes should be expected, in view of the fact that the cross mut. *formosa* × f. *typica* strain C mendelizes and gives a monohybrid ratio in the F_2 generation.

As has already been discussed, certain irregularities occur during the heterotypic metaphase, through the deviation of the chromosomes from the characteristic zigzag arrangement, which sometimes result in the abnormal distribution of the chromosomes to the two poles, eight chromosomes going to one pole and six to the other instead of seven and seven. In certain cases, even though the number of chromosomes distributed to each of the two poles is normal, the paternal and the maternal set exchange a chromosome or chromosomes. This gives an abnormal result in breeding. The pollen grain with eight chromosomes may possibly function and give rise to fifteen-chromosomed individuals. The pollen grains with six chromosomes probably do not function at all, as no species have been discovered so far with only thirteen chromosomes. These two cases will be omitted entirely from the discussion that is to follow. The complexes that can arise through an irregularity, even though the distribution of the number of chromosomes to the two daughter nuclei is normal, are many and varied, depending on the nature of the irregularity, the point at which it occurred, and its relation to individual chromosomes, etc. These irregularities have been thoroughly discussed by CLELAND (18, 19). He has also calculated the

theoretical expectancies and the genetical results arising therefrom in case of some such complexes.

From the results of her breeding experiments, FRIEDA COBB BLANCHARD has formed a hypothesis of the constitutions of *O. pratincola* and its revolute-leaved mutations. Her paper dealing with this problem will probably precede the present paper in publication. The hypotheses which she offers have been made known to the writer, and he has adopted them in explanation of his cytological findings. Her explanation of the origin of mut. *formosa* is given in the following paragraph.

Let 1, 2, 3, 4, 5, 6, 7 represent the chromosomes of the α gamete of *O. pratincola* strain E and 8, 9, 10, 11, 12, 13, 14 represent the chromosomes of the β gamete. Then the zygote formed will have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 as its chromosome constitution. This zygote in reduction division will in turn produce α gametes having chromosomes 1, 2, 3, 4, 5, 6, 7 and β gametes having 8, 9, 10, 11, 12, 13, 14. Now suppose an irregularity in the reduction division to occur in the production of the eggs, and chromosome 7 of the α gamete to dissociate from the other members of its series and to become interchanged with chromosome 14 of the β series, so that chromosome 7 goes to the β complex and chromosome 14 to the α complex. In such a case we shall have 1, 2, 3, 4, 5, 6, 14 for the α gamete and 8, 9, 10, 11, 12, 13, 7 for the β gamete. (For an explanation of the conception of this type of a mutation see the section entitled "Whole-chromosome crossovers" in BLANCHARD and BARTLETT 8.) If this modified α egg is fertilized by a regular sperm of *O. pratincola* strain E having 8, 9, 10, 11, 12, 13, 14 as its chromosome constitution, the zygote will have the constitution 1, 2, 3, 4, 5, 6, *14*, 8, 9, 10, 11, 12, 13, *14*. There will be a pair of identical chromosomes in this combination. BLANCHARD believes that mut. *formosa* arises by such a whole chromosome cross-over, the chromosome which becomes duplicated being that one of the β complex of strain E which carries the recessive factor for revolute leaves. If this assumption is correct, mut. *formosa* might be expected to show a pairing of two of the chromosomes during the final prophase stages (diakinesis). Apparently, however, there is no such free pair, but it is conceivable that a pair of homologous chromosomes might not

be able to break away from the closed chain. Several others of the revolute-leaved mutations of strain E are probably the results of whole chromosome crossing-over of the type just discussed, where one or more chromosomes from one complex exchange their places with chromosomes of the other complex.

Linkage in *Oenothera* has been the subject of some discussion in recent literature. SHULL (78, 79) thinks that, except for the *brevistylis* and old gold (80) characters, all the known factors of *Oenothera* probably lie in a single chromosome pair. In this pair SHULL believes that he has located nine factors, affecting visible characters, with which two gametic and two zygotic lethals are supposed to be associated. EMERSON (31) has shown that the data offered by SHULL in support of this hypothesis have some discrepancies, and that they do not establish the correctness of the hypothesis.

It appears that linkage in *Oenothera* is due to the cohesion of the chromosomes, and their separation in the heterotypic division in such a way that the maternal set goes to one pole and the paternal set to the other pole of the spindle. It is probable that in *O. pratincola* the maternal and paternal members of the chromosome set alternate with each other, and the characteristic zigzag arrangement at the heterotypic metaphase effects their separation; thus the paternal and maternal sets of chromosomes come out as they enter the zygote. Hence in *O. pratincola* the characteristic group of α chromosomes behaves as a single chromosome and is responsible for a group of linked characters. Interchange of a whole chromosome or chromosomes between the α and the β complex does occur as a result of an irregularity in the zigzag arrangement of the chromosomes during the heterotypic metaphase. When such an interchange of chromosomes takes place (a "whole chromosome cross-over") it probably gives rise to a mutation. One case of this type has already been discussed.

GATES (46) holds that partial linkage and crossing over probably cannot occur in *Oenothera* because of the telosynaptic arrangement of the chromosomes. Due to such an arrangement, the chromosomes cannot twist about one another during the strepsenema stage and exchange chromatin material, as they do in most plants; hence "crossing over" may not always be possible. CLELAND (18) observes

the appressed sides in one or more peripheral loops during late stages of second contraction. These, he thinks, have apparently become twisted about each other in a way that might conceivably result in the exchange of chromatin particles. He thinks crossing over of this type is possible. HÅKANSSON (53) also thinks that there is a possibility of crossing over occurring in the second contraction. SHULL (80) maintains that the genetical evidence of linkage and crossing over is unequivocal in his cultures. He observes new and striking examples of it every season. *O. pratincola*, which has been under cultivation and has furnished material for the genetical researches of BARTLETT and BLANCHARD for the last fifteen years, does not as yet show any crossing over which cannot be explained by whole chromosome crossing over.

Summary

1. The diploid chromosome number in *Oenothera pratincola* is fourteen. The chromosomes at metaphase of the somatic mitosis are rod-shaped, variously bent, and are formed by the cross segmentation of the spireme.

2. The resting nuclei of the pollen mother cells are characterized by a network of delicate threads. Chromatin granules varying in size are scattered through the chromatic network, but more thickly at the periphery.

3. There is one large nucleolus and some smaller nucleoli. All of these bodies are in intimate contact with the chromatic network, and probably contribute their material to the spireme developed during the heterotypic prophase. An endonucleolus is present and may be observed from synizesis on to the heterotypic metaphase. The nucleolus is believed to be a storage organ for material related to chromatin, which is drawn upon in the development of the spireme during the heterotypic mitosis.

4. The approach of the heterotypic prophase is indicated by occasional parallel arrangements of the chromatic threads, but these are not very conspicuous. A gradual thickening of the threads is brought about by condensation and the movement of chromatic material along the threads. There is no indication of the fusion of two parallel threads at this stage.

5. As the nucleus enters the prophase, the threads attached to the nuclear membrane seem to contract, probably through condensation, and become gathered to form a ball of threads, the synizetic knot.

6. During synizesis many threads disappear, having delivered their chromatin contents to the threads which are to survive. These become prominent and uniform, and a spireme arises as the knot gradually expands. Some parallelisms are also to be noticed during synizesis, but these are certainly due to the irregular contraction and condensation that take place. Conditions indicate that there is no association or fusion of the separated halves of a univalent spireme or of two univalent spiremes to form a bivalent spireme.

7. The open spireme stage results from the gradual expansion of the synizetic knot, and presents threads of almost uniform thickness throughout the spireme. No free ends were observed in the thread system.

8. By the further contraction and condensation of the threads of the open spireme, the stage known as the second contraction is reached. During this stage all the chromatic material is gathered at the center of the nucleus, the threads in the center thickening more rapidly. Loops of the threads are not lost, even during the stage of greatest contraction, but they become much smaller and radiate from the central mass of chromatic material. These loops are uniformly single threads.

9. The loops could be traced throughout all stages of second contraction, and undoubtedly give rise in part to the univalent chromosomes which emerge at the end of the second contraction. The univalent chromosomes are arranged end to end, that is, telosynaptically.

10. At the stage known as diakinesis in most plants and animals, a closed chain of fourteen chromosomes attached end to end is found in *O. pratincola* strain E, which convincingly points to a telosynaptic association. Occasionally the closed chain breaks at some point to form an open chain of fourteen chromosomes.

11. The chain of chromosomes passes to the equatorial region of the spindle, where the spindle fibers become attached in such a way that alternate chromosomes pass to the same pole. There is a characteristic zigzag arrangement of the chromosomes at metaphase.

12. Anaphase is mostly normal, seven chromosomes passing to each pole; but there are about 3 to 7 per cent of exceptions, due to irregularities in the zigzag arrangement of the chromosomes which result in six chromosomes passing to one pole and eight to the other.

13. A lengthwise split of the chromosomes in preparation for the homeotypic mitosis is effected in late telophase of the heterotypic division.

14. During interkinesis the halves of the split chromosomes (daughter chromosomes) are connected in their middle region; but the ends are widely separated so that each pair resembles an X.

15. The homeotypic divisions are regular and occur simultaneously. The two spindles lie sometimes in the same plane and sometimes at right angles to each other.

16. All of the stages of meiosis in the pollen mother cells of *O. pratincola* strain C are identical with those of *O. pratincola* strain E and of its mut. *formosa*.

17. Strain M (*formosa*×strain C) agrees with *O. pratincola* strain E, strain C, and mut. *formosa* in all the stages of meiosis, up to and including second contraction. Following second contraction, in strain M there emerges a closed chain of twelve chromosomes, with a ring of two attached to it. The ring of two chromosomes breaks away from the closed chain of twelve at the heterotypic metaphase, and the two chromosomes of the pair pass to different poles.

18. This pair of chromosomes is significant of the genetic behavior of strain M. The chromosomes are probably homologous and carry the genes for flat leaf and revolute leaf, and their segregation and recombination in the breeding of strain M give the simple Mendelian ratio.

19. The grand-daughter nuclei pass into a resting condition in which the outlines of the chromosomes become lost in the chromatic network. The process is one of expansion and irregular distribution of the chromatin throughout the nucleus.

20. The segmentation of the protoplasm to form the four pollen grains is effected by furrows which start at the periphery of the pollen mother cell (between the four nuclei) and grow inward, cutting through regions of vacuoles as they pass to the center of the cell.

21. The position of the chromosomes in the chain is believed to be fixed, the maternal alternating with the paternal.

22. The failure of the chromosomes to pair in the late prophase of the heterotypic mitosis is probably because of unlike genetical constitution, but the hypothesis that the formation of chains in certain species of *Oenothera* is an indication of their hybridity is believed to be untenable.

23. *O. pratincola* produces two types of gametes, called α and β. Generally the functional egg is an α gamete and a functional sperm a β gamete.

24. Irregularities in chromosome distribution during the heterotypic metaphase result at times in an exchange of chromosomes, so that an α chromosome enters a β complex and vice versa, and such interchange of chromosomes is probably the origin of certain mutations. By the theory of FRIEDA COBB BLANCHARD, presented in the genetical discussion, the origin of mut. *formosa* from *O. pratincola* strain E is due to such an interchange of chromosomes.

25. Certain examples of linkage in *Oenothera* are probably due to cohesion of non-homologous chromosomes in groups. Crossing-over has not been observed in *Oenothera pratincola*.

It is a pleasure to acknowledge indebtedness to Professor H. H. BARTLETT and Dr. FRIEDA COBB BLANCHARD for allowing me to collect material from their cultures, and also for advice on the genetical phase of the work. I wish to express thanks to Professor B. M. DAVIS also, for his criticism of cytological conclusions and for his kindness in offering many facilities.

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EXPLANATION OF PLATES VII–IX

All figures were sketched with the aid of a camera lucida, under a Spencer microscope, with Spencer 1.8 oil-immersion objective and 18× eye-piece; they have been reduced here one-fourth in reproduction; present magnification approximately 1400 diameters. *Oenothera pratincola* strain E unless otherwise stated.

PLATE VII

Fig. 1.—Metaphase of mitosis in archesporial cell viewed from pole of spindle, showing fourteen chromosomes.

Fig. 2.—Early anaphase of mitosis in archesporial cell.

Fig. 3.—Resting nucleus of pollen mother cell.

Fig. 4.—Gradual thickening of threads, with occasional parallelism, approaching prophase of heterotypic mitosis.

Fig. 5.—Advanced stage in thickening of threads, giving a coarse reticulum.

Fig. 6.—Beginning of contraction of reticulum prior to synizesis; threads varying greatly in thickness.

Fig. 7.—Early synizesis showing irregular thickening of threads.

Fig. 8.—Mid-synizesis.

Fig. 9.—Loosening of synizetic knot; threads somewhat thicker and more uniform.

Fig. 10.—Open spireme emerging from synizesis; note mass of contracted threads at left.

Fig. 11.—Open spireme, single threads.

Fig. 12.—Open spireme, thread showing chromomeres.

Fig. 13.—Second contraction; note thickening of threads in center and peripheral loops.

Fig. 14.—Second contraction, showing thick threads just before differentiation of chromosomes.

Fig. 15.—Second contraction; three chromosomes distinct.

Fig. 16.—Second contraction; chromosomes evident.

PLATE VIII

FIG. 17.—End of second contraction stage; chromosomes distinct but still crowded.

FIGS. 18–20.—Closed chains of fourteen chromosomes in late prophase.

FIG. 21.—Open chain of fourteen chromosomes in late prophase due to break in one of attachments.

FIG. 22.—Closed chain of fourteen chromosomes; multipolar spindle omitted.

FIG. 23.—Open chain of fourteen chromosomes; multipolar spindle.

FIG. 24.—Heterotypic metaphase showing regular zigzag arrangement of chromosomes; alternate chromosomes go to same pole.

FIG. 25.—Two chromosomes (*a*, *b*) in different parts of chain suspended between the poles.

FIG. 26.—Two pairs of adjacent chromosomes (*a*, *b*) passing to different poles of spindle.

FIG. 27.—Pair of adjacent chromosomes (*a*) passing to one pole with single chromosome (*b*) suspended on equatorial plate.

FIGS. 28–30.—Chromosome groups with fiber attachments so irregular that distribution of chromosomes is uncertain.

FIG. 31.—Late anaphase, polar views.

Fig. 32.—Late anaphase, showing unequal distribution of chromosomes (6 and 8).

PLATE IX

Fig. 33.—Late anaphase, polar view, showing typical arrangement of chromosomes.

FIG. 34.—Interkinesis, showing split chromosomes and formation of new nucleoli.

FIG. 35.—Interkinesis, showing X-like pairs of daughter chromosomes with denser regions.

FIG. 36.—Homeotypic prophase with pairs of daughter chromosomes now condensed; an example of irregular distribution, six chromosomes in one nucleus and eight in other; note prominent split in chromosomes.

FIG. 37.—Homeotypic metaphase; side view of one plate and polar view of other, showing regular distribution of chromosomes.

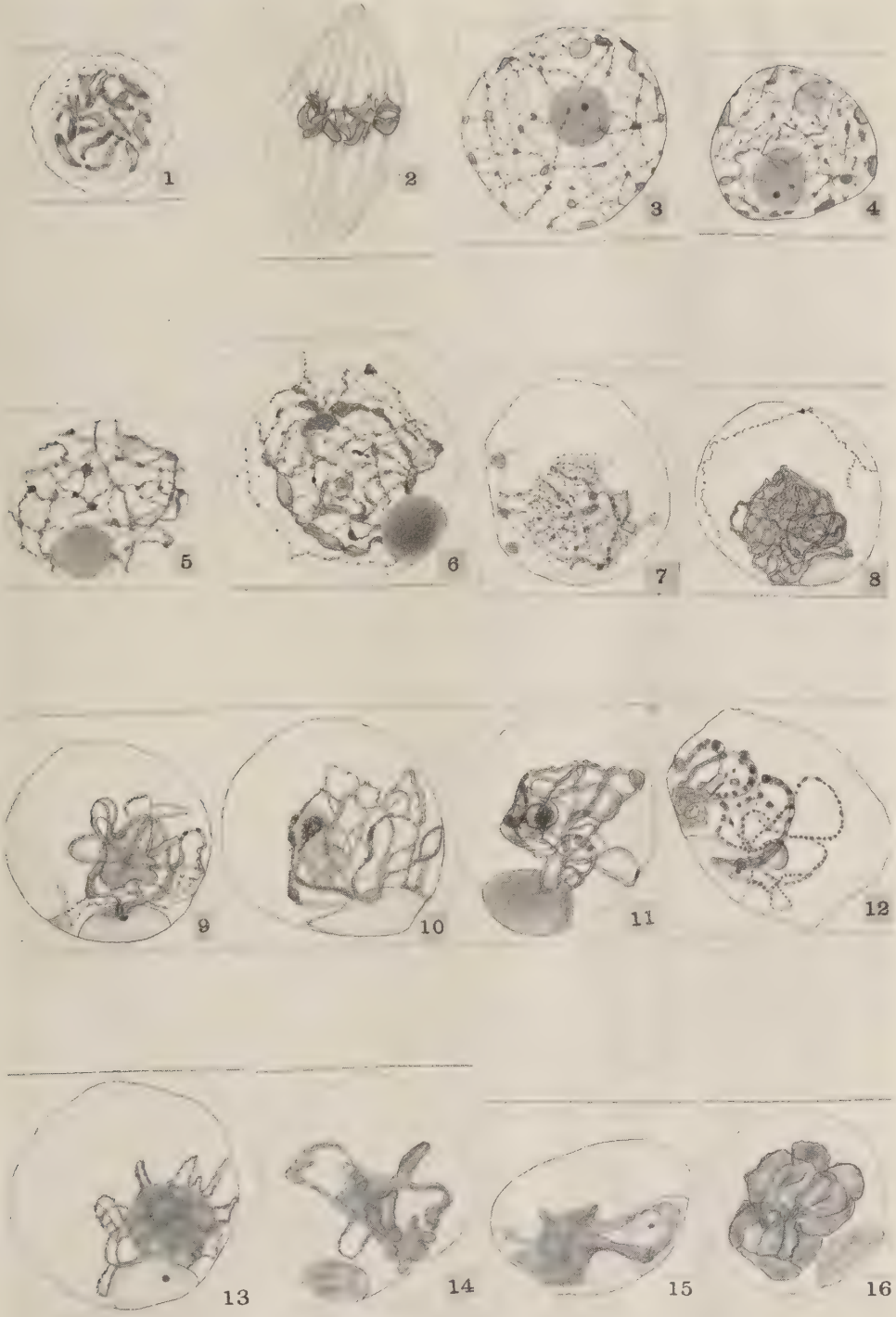
FIG. 38.—Telophase of homeotypic mitosis, chromosomes spun out into threads forming irregular network.

FIG. 39.—Closed chain of fourteen chromosomes in mut. *formosa;* late prophase.

FIG. 40.—Open chain of fourteen chromosomes in mut. *formosa*, result of break in chain; late prophase.

FIG. 41.—Closed chain of twelve chromosomes and ring of two (*a*) in strain M; late prophase.

FIG. 42.—Closed chain of twelve chromosomes and ring of two (*a*) in strain M; multipolar spindle omitted.



KULKARNI on OENOTHERA

FIG. 43.—Same as fig. 42.

FIG. 44.—Metaphase of heterotypic mitosis in strain M; chain of twelve chromosomes and ring of two (*a*) at side of spindle separate from chain.

FIG. 45.—Late telophase of homeotypic mitosis; boundaries of some of chromosomes still visible; note spindle fibers connecting nuclei.

FIG. 46.—Late telophase; small refractive vacuoles in cytoplasm; wall of pollen mother cell infolded between nuclei.

FIG. 47.—Large flattened vacuoles formed by fusion of vacuoles between nuclei from center of pollen mother cell to furrows at periphery.

FIG. 48.—Furrows cutting inward from periphery toward center of pollen mother cell.